國 立 交 通 大 學

National Chiao Tung University

生物資訊研究所

碩 士 論 文

基於支持向量機器方法之蛋白質β-turn預測

Prediction of β-Turns in Proteins with Support Vector Machines

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中 文 摘 要

本研究是利用支持向量機器的方法來預測蛋白質中β-turn的位置。在僅有蛋白質之胺基酸序列的情況下找尋有用的特徵向量，並將這些資訊輸入支持向量機器中，以此方法來預測蛋白質中哪些殘基會形成β-turn。本研究使用 426 條非同源蛋白質，做 7 倍的交叉認證以驗證預測的準確率。由結果發現除了前人研究提及的多重序列比對及二級結構資訊外，殘基暴露於溶劑的程度亦可提供有用的資訊；而胺基酸的體積及親水程度則對β-turn的預測無明顯助益。

本研究整合了多重序列比對所產生的位置加權矩陣，二級結構預測資訊，以及殘基暴露於溶劑之程度的預測等三種特徵向量，則總準確率可達 79.6%，MCC值可達 0.48，皆高於其他β-turn預測方法。

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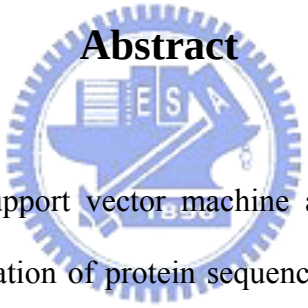


Abstract

In this study, we use support vector machine approach to predict β-turns in protein. With only the information of protein sequence, we try to find useful feature vectors based on amino acid, and import the information to SVM to predict which residue would be in β-turn. We use 426 non-homologous proteins as dataset, and 7-folded cross validation to examine the prediction performance. In addition to multiple sequence alignment and secondary structure information, we found that relative solvent accessibility could also provide useful information in β-turn prediction.

In this work, import multiple feature vectors of multiple sequence alignment information (PSSM), secondary structure prediction, and relative solvent accessibility prediction, the Q_{total} could reach 79.6% and the MCC value is 0.48. Both these two measure performance are better than other previous methods.

目錄

1. Introduction

The knowledge of the secondary structure of a protein has great importance in the study of the protein functionality. Currently, the main technique to determine protein structure is X-ray crystallography, which is a slow, and often a difficult process. On the other hand, the protein sequence data arising from sequencing projects is growing rapidly. Thus, it is increasingly important to predict the structure of proteins whose sequences are known. One significant step towards elucidating the structure and function of a protein is the prediction of its secondary structure.

Protein secondary structure is characterized by regular elements such as α-helices and β-sheets and non-repetitive motifs such as tight turns, bulges and random coil structures. A tight turn in protein structure is defined as a site where a polypeptide chain reverses its overall direction, i.e., leads the chain to fold back on itself by nearly 180°, and the amino acid residues directly involved in forming the turn are no more than six. Depending on the number of residues forming the turn, tight turns are further classified as δ, γ, β, α, and π-turns [1]. Among the tight turns, β-turn is the most predominant one. A β-turn involves four amino acid residues. The β-turns originally recognized by Venkatachalam [2] are stabilized by a hydrogen bond between the backbone CO(i) and the backbone NH(i + 3). However, Lewis *et al.* [9] found that 25% of β-turns are "open," i.e., have no intra-turn hydrogen bond at all as stipulated by Venkatachalam [2]. Open turns do not lend themselves to classification by dihedral angles. Therefore, the definition widely accepted for b-turns is: A β-turn comprises four consecutive residues where the distance between $C_\alpha(i)$ and $C_\alpha(i + 3)$ is less than 7 Å, and the tetrapeptide chain is not in a helical conformation. The distance between the C_α atoms in the first and last residues of a tetrapeptide, i.e., $C_\alpha(i)$ and $C_\alpha(i + 3)$, is a

key criterion common to all β-turns, further the backbone dihedral angles in the inner residues $i + 1$ and $i + 2$ will define different types of β-turns.

On average, about 25% of all protein residues comprise β-turns [5]. As one of the most common types of non-repetitive motifs in proteins, β-turns bear great significance in protein structure and function. Both from structural and functional point of view, β-turns play important biological roles as reflected from the following points: First, β-turns are four-residue reversals in proteins so that they help in the formation of higher-order structure [6]. A polypeptide chain cannot fold into a globular fold without β-turns; Second, β-turns usually occur on the exposed surface of a protein and are likely to be involved in molecular recognition processes and interactions between receptors and substrates [1; 4], and provide very useful information for designing template structures for the design of new molecules such as drugs, pesticides, and antigens. Furthermore, being at solvent-exposed surfaces, the residues that form β-turns tend to be hydrophilic residues.; and third, also play an important role in protein folding and stability [6]. Further, one major secondary structural feature of many biologically active peptides is β-turn. β-Turn forms an integral component in the fundamental building block for anti-parallel β-sheets, which plays a good candidate for molecular recognition processes since being at solvent-exposed surfaces, and its formation is an important stage during the process of protein folding [6]. Therefore, to improve on the identification of structural motifs such as the building block for anti-parallel β-sheets and fold recognition, an accurate method to identify the location of β-turns in a protein sequence needs to be developed. Consequently, prediction of β-turns would be small step toward the overall prediction of three-dimensional structure of a protein from its amino acid sequence. It will also help in identification of structural motifs such as β-hairpin. β-turns provide very useful information for

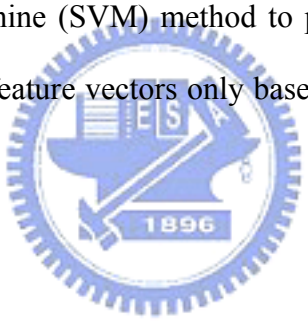
defining template structures for the design of new molecules such as drugs, pesticides, and antigens.

A number of β-turn prediction methods have been developed, they can be divided into two categories: statistics-based and machine learning-based methods. The majority of statistics-based methods empirically employed the 'positional preference approaches" [7; 8; 9; 10; 11]. In the Chou–Fasman method [8], a set of probabilities is assigned to each residue and the conformational parameters and positional frequencies are determined by calculating the relative frequency of each secondary structure. In the 1–4 and 2–3 correlation model [11], the coupling effects between the first and fourth residues and between the second and third residues are taken into account. In the sequence coupled model developed by Chou [7] within the first-order Markov chain framework, the sequence correlation effect for an entire oligo-peptide is considered. GORBTURN uses the positional frequencies and equivalent parameters [12] to remove the potential helix and strand forming residues from the β-turn prediction [13]. As to machine learning-based methods, a neural network method, BTPRED, was developed by Shepherd et al. [14] to predict the location and type of β-turns in proteins. The prediction performance could not be objectively compared because of the different dataset in these methods. Kaur and Raghava evaluated these methods and found that BTPRED was most accurate among these β-turn prediction methods [15].

BetaTPred2, an improved neural network method was developed by Kaur and Raghava [16]. In that method, they use multiple sequence alignment as input instead of the single amino acid sequence, and a great improvement in prediction performance has been achieved (Matthews correlation coefficient MCC = 0.43). *k*–nearest neighbor method, which is combined with a filter that uses predicted protein secondary

structure information was developed by Kim [17].

The SVM is an extremely successful learning theory that usually outperforms other machine learning technologies such as artificial neural networks (ANNs) and nearest neighbor methods. In recent years, SVMs have performed well in diverse applications of bioinformatics in several aspects including prediction of secondary structure [18; 19], classification of protein quaternary structure [20], etc. In this work, we attempt to predict β-turns in proteins using support vector machine (SVM) with various information derive from protein sequence, comparing with some other β-turn prediction methods that were recently evaluated by Kaur and Raghava [15], and with the other β-turn prediction methods that using the same data set. In this study. we employ a support vector machine (SVM) method to predict β-turns in proteins, and attempt to seek helpful input feature vectors only based on the information of protein sequences.



2. Material and methods

2.1 The dataset

In this study, The dataset is comprised of 426 non-homologous protein chains which were first described by Guruprasad and Rajkumar [21]. This same dataset was selected by Kaur and Raghava [15] to evaluate the performance of six β-turn prediction methods. In this dataset, any two protein chains have $\leqq$ 25% sequence identity. The structure of these proteins is determined by X-ray crystallography at better than 2.0 Å resolution, and each protein chain contains at least one β-turn . The program PROMOTIF [22] has been used to assign β-turns in proteins.

2.2 Cross validation method

A prediction method is often developed by cross-validation or jack-knife method [23]. In a full jack-knife test of N proteins, one protein is removed from the set, the training is done on the remaining N−1 proteins and the testing is done on the removed protein. This process is repeated *N* times by removing each protein in turn. Because of the size of the data set, the jack-knife method would be very time consuming, so a more limited cross-validation has been used. In this study, a 7-fold cross-validation technique is used where the data set is randomly divided into 7 subsets, each containing equal number of proteins. The training set is consisted of 6 subsets, and tested the prediction performance on the excluded set, the testing set. This has been done seven times to test for each subset. The final prediction results have been averaged over seven testing sets.

2.3 The support Vector Machine (SVM)

The SVM is a technique of machine learning based on statistical learning theory [24]. SVM has recently be applied to solve the problems in Bioinformatics, such as secondary structure prediction [18; 19], fold recognition, etc. A library for support vector machines（LIBSVM）is used in this study [25], and which is an implementation of SVM in C language for the problem of classification, and kernel type is radial basis function.

The basic idea of SVM to pattern classification can be stated briefly as follow two steps. First, map the input vectors into a feature space (often with a higher dimension), either linearly or non-linearly, which is relevant with the selection of the kernel function. Second, classifying the data by seeking an optimal separating hyperplane which can maximize the distance between two classes.（Figure 1） SVM training always seeks a global optimized solution and avoids over-fitting, so it has the ability to deal with a large number of features. A complete description of the theory of SVMs for pattern recognition has been done by Vapnik [26].

Two parameters were adjusted for optimal performance. In this work, we employed the radial basis function kernel as the kernel function. (Eq. 1) The first parameters to be determined are γ and the regularization parameter C. In the present case, we set γ=0.0625, C=2.

$$K(X_i, X_j) = \exp(-\gamma(X_i - X_j)^2) \quad (1)$$

2.4 The input feature vectors

Several different input feature vectors for the support vector machine（SVM） are considered. We use the classical local coding scheme of the protein sequences with a sliding window. In this study, the window size was set as 9. The “null” residue was added in order to allow a window to extend over the N- and the C-terminus.

2.4.1 Sequence input vector

The amino acid type of each residue is encoded into a 20-dimension vector consist of 19 “0” and single “1”, e.g. glycine is represented as (1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0), alanine is represented as (0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0), etc. The “null” residue is represented by a 20-dimension vector of all-zero.

2.4.2 Multiple sequence alignment (PSSM) input vector

With multiple sequence alignments, we use PSI-BLAST to detect distant homologues of a query sequence and generate position-specific scoring matrix (PSSM). The matrix has 21 × M elements, where M is the length of the target sequence, and each element represented the likelihood of that particular residue substitution at that position [27]. These profiles were scaled to 0-1 range using the standard logistic function:

$$f(x) = \frac{1}{1 + \exp(-x)} \tag{2}$$

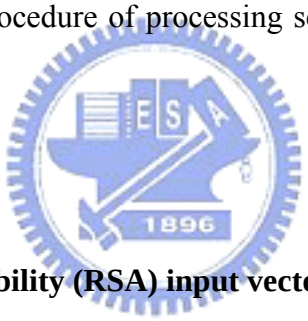
The “null” residue is represented by a 20-dimension vector of all-zero.

A figure introduce the procedure of processing PSSM input vector was shown in Figure 2.

2.4.3 Secondary structure (SS) input vector

The secondary structure of each proteins in the dataset was predicted by PSIPRED [28]. The PSIPRED prediction could output the probabilities of three states secondary structure (helix, strand, coil). Because of the value was in the range from 0-1, the three probabilities of each residue could directly be used as 3-dimension vector. The "null" residue is represented by a 3-dimension vector of all-zero.

A figure introduce the procedure of processing secondary structure input vector was shown in Figure 3.



2.4.4 Relative solvent accessibility (RSA) input vector

Amino acid solvent accessibility is the degree to which a residue in a protein is accessible to a solvent molecule. Relative solvent accessibility was calculated by dividing the DSSP-defined solvent accessibility by the accessibility for a Gly-X-Gly tripeptide given by the method of Rose and Dworkin [29]. In this study, we use Jnet [30] to predict relative solvent accessibility of each residue based on a two state-model (exposed/buried) in three categories: 25%, 5%, and 0% accessible. Each residue was represented as 3-dimension vector (RSA-3) composed of zero and one, "one" means the residue was predicted as exposed at each accessible class, and "zero" means buried. The "null" residue is represented by a 3-dimension vector of all-zero.

Furthermore, we use another relative solvent accessibility prediction method

developed by our lab members [36], which could predict two-state RSA in ten categories: 0-10%, 10-20%, …, 90-100%. With this RSA prediction scheme, the predicted probabilities of the ten classes were available, and they were directly be used as 10-dimension vector (RSA-10). The "null" residue is represented by a 10-dimension vector of all-zero.

A figure introduce the procedure of processing RSA-10 input vector was shown in Figure 4.

2.4.5 Chou-Fasman conformational parameter input vector

The Chou-Fasman algorithm for the prediction of protein secondary structure [31] is one of the most widely used predictive schemes. The Chou-Fasman method of secondary structure prediction depends on assigning a set of prediction values to a residue and then applying a simple algorithm to the conformational parameters and positional frequencies (Table 1.).

The Chou-Fasman algorithm is simple in principle. The conformational parameters for each amino acid were calculated by considering the relative frequency of a given amino acid within a protein, its occurrence in a given type of secondary structure, and the fraction of residues occurring in that type of structure. These parameters are measures of a given amino acid's preference to be found in helix, sheet or coil. Using these conformational parameters, one finds nucleation sites within the sequence and extends them until a stretch of amino acids is encountered that is not disposed to occur in that type of structure or until a stretch is encountered that has a greater disposition for another type of structure. At that point, the structure is terminated. This process is repeated throughout the sequence until the entire sequence is predicted.

In order to scale the conformational parameters to 0-1, each value of parameter was simply divided by 200. Each amino acid was represented as 3-dimension vector of its scaled conformational parameters. The “null” residue is represented by a 3-dimension vector of all-zero.

2.4.6 Amino acid solvent exposed area (SEA) input vector

Table 2. implies solvent accessibility information derived from Bordo and Argos [32]. The data for this table was calculated from data taken from 55 proteins in the Brookhaven data base, coming from 9 molecular families: globi ns, immunoglobins, cytochromes c, serine proteases, subtilisins, calcium binding proteins, acid proteases, toxins and virus capsid proteins. Red entries are found on the surface of a proteins on > 70% of occurrences and blue entries are found inside of a protein of < 20% of occurrences. The only clear trend in this table is that some residues, such as R and K, locate themselves so that they have access to the solvent. The hydrophobic residues, such as L and F, show no clear trend: they are found near the solvent as often as they are found buried. Each amino acid was represented as 3-dimension vector and the “null” residue is represented by a 3-dimension vector of all-zero.

2.4.7 Amino acid volume input vector

We use the amino acid volume calculated by Zamyatnin [33] as one kind of input vectors. As shown in Table 3., each amino acid was represented by its volume as one-dimension vector, and the “null” residue is represented by an one-dimension vector of zero.

2.4.8 Amino acid hydropathy input vector

Hydropathy index listed a scale combining hydrophobicity and hydorphilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (- values) or a hydrophobic environment (+ values) [34]. As shown in Table 4., and the value was scaled to 0-1 by equation (2). Each amino acid was represented by its hydropathy as one-dimension vector. and the "null" residue is represented by an one-dimension vector of zero.

2.5 Adjusting the threshold of the output of LIBSVM

The SVM tool we used in this study (LIBSVM) could estimates the probability of each predicted class as setting parameter b as 1. In β-turn prediction, there are only two classes labels: turn and non-turn. If the probability of residues which was predicted as β-turn is more than 0.5, the output label would be β-turn, otherwise the predicted output label would be non-turn. But it seems that the threshold 0.5 is to high to get good sensitivity, so we try to lower the threshold to seek better prediction performance.

2.6 Filtering

The prediction is performed for each residue separately, since β-turns are typically multiple turns of at least four residues long, we added a simple filtering step which is similar to the "state-flipping" rule used in Shepherd *et al*. [14]. A set of five rules have been used in the following order:

1. Flip isolated nonturn predictions to turn (i.e., t-t → ttt).

2. Flip isolated pairs of nonturn predictions to turn (i.e., t--t →. tttt).

3. Flip isolated turn predictions to nonturn (i.e., -t- → ---).

4. For isolated pairs of turn predictions, flip the adjacent nonturn predictions to turn (i.e., -tt- → tttt).

5. For isolated triplet of turn predictions, flip the adjacent nonturn predictions to turn (i.e., -ttt- → ttttt).

2.7 Performance measures

Several parameters were widely used to measure the performance of β-turn prediction methods as described by Shepherd et al. [14], which are based on the following scalar quantities:

p, the number of correctly classified β-turn residues

n, the number of correctly classified non-β-turn residues

o, the number non-b-turn residues incorrectly classified as β-turn (over-predictions)

u, the number b-turn residues incorrectly classified as non-β-turn (under-predictions)

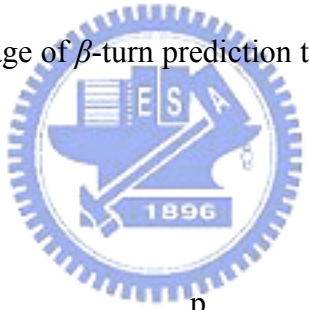
t , the total number of residues.

The parameters were described below.

1. **Qtotal** (or prediction accuracy), the percentage of correctly classified residues. It is the most common measure of a method's overall performance; however, *Q*total can be misleading as *β*-turn residues occur much less frequently than non-*β*-turn residues in proteins (25 versus 75%). Therefore, one could easily achieve *Q*total = 75% merely by predicting all residues to be non-*β*-turn.

$$Q_{toaal} = \frac{p+n}{t} \times 100 \quad (3)$$

2. **Qpredicted** is the percentage of *β*-turn prediction that are correct, which penalizes over-predictions.



$$Qpredicted = \frac{p}{p+o} \times 100 \quad (4)$$

3. **Qobserved** is the percentage of observed *β*-turns that are correctly predicted, which penalizes under-predictions.

$$Qobserved = \frac{p}{p+u} \times 100 \quad (5)$$

4. **MCC** (Matthew's Correlation Coefficient), a single measure of performance that takes into account for both over- and under-predictions.

$$\mathrm{MCC} = \frac{pn - ou}{\sqrt{(p+o)(p+u)(n+o)(n+u)}} \tag{6}$$

3. Results

SVM is used predict the β-turns in the proteins, and it needs to import useful information. The information, which represents β-turn, is taken from a protein sequence; it can be coded through several ways and is called a feature vector.

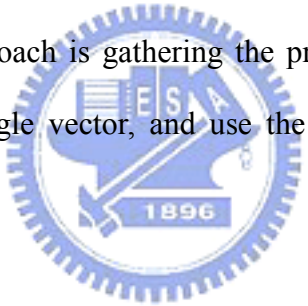
3.1 Prediction accuracies of using single feature vector

Nine feature vector was used individually to predict β-turns in protein, and the prediction performance was listed in Table 5. As shown in Table 5., comparing the prediction performance of each input feature vector, the multiple sequence alignment feature vector which encoded PSSM as import information could achieve the best result with MCC value of 0.46 and the total accuracy (Q_{total}) of 76.4. Secondary structure feature vector could also get a good prediction performance with MCC of 0.42 and Q_{total} of 72.9. Both RSA-3 and RSA-10 in Table 5. mean relative solvent accessibility feature vector: RSA-3 indicates utilizing three classes of solvent accessibility prediction from Jnet; and RSA-10 denotes employing another ten-classes relative solvent accessibility prediction approach. RSA-10 shown better prediction effect than RSA-3 in all respects: Q_{total} raise from 69.4% to 72.9%, $Q_{predicted}$ raise from 41.7% to 46.5%, $Q_{observed}$ raise from 62.3% to 69.8%, and MCC raise from 0.30 to 0.39. The predict performance of importing Chou-Fasman conformational parameter and sequence are similar. With Chou-Fasman conformational parameter feature vector, Q_{total}, $Q_{predicted}$, $Q_{observed}$, and MCC are 72.7%, 44.9%, 48.1%, and 0.28 respectively. As to the performance of using sequence as input feature vector, Q_{total}, $Q_{predicted}$,$Q_{observed}$, and MCC are 71.9%, 44.2%, 54.4%, and 0.30 respectively. With the solvent exposed area (SEA) feature vector, it could get a good sensitivity yielded

$Q_{observed}$ of 72.8%, which is nearly the same as the $Q_{observed}$ value of multiple sequence alignment feature vector; but the correct prediction rate is much lower with the $Q_{predicted}$ value of 26.6%; hence the MCC value and Q_{total} are only 0.07 and 44.1% respectively. The prediction performance of SEA feature vector, volume feature vector, and hydorpathy feature vector is much lower than other feature vectors with MCC value of 0.07, 0.06, and 0.12 respectively.

3.2 Assembling feature vectors

In this study, we employ two strategies to assemble the information of each single feature vectors. One is encoding multiple feature vectors in a SVM training and testing data file; another approach is gathering the probability of being β-turn from each prediction output of single vector, and use the probabilities as import of the second layer SVM prediction.



3.2.1 Prediction accuracies of using Multiple feature vectors

As shown if Table 5., multiple sequence alignment feature vector could receive better prediction performance than other feature vectors, thus we take it as leading role, and try to join other feature vectors as import. Because of the worse prediction performance of SEA feature vector, volume feature vector, and hydorpathy feature vector, these feature vectors were not be considered in this coding scheme. The results of multiple feature vectors were listed in Table 6.

In this method, all the multiple feature vectors listed in Table 6. could slightly raise Q_{total}, but not every combination is fessible to improve MCC. With slightly arise

$Q_{predicted}$ but lower $Q_{observed}$, MCC value of PSSM+SEA-3, PSSM+SEA-10, and PSSM+C-F are the same or worse than MCC of PSSM. As to compare the prediction performance of importing two feature vectors, PSSM+SS, to single vector of PSSM, Q_{total} is raise from 76.4% to 78.2%, $Q_{predicted}$ is raise from 51.4% to 54.3%, and MCC value is raise from 0.46 to 0.48, but $Q_{observed}$ is slightly lower from 72.8% to 71.9%. The performance of importing three feature vectors, PSSM+SS+SEA-3, is also better than single vector of PSSM, Q_{total} is raise from 76.4% to 79.6%, and MCC value is raise from 0.46 to 0.48.

3.2.2 Prediction accuracies of performing Double-layer SVM

As listed in Table 5., multiple sequence alignment feature vector, secondary structure feature vector, and ten-classes relative solvent accessibility feature vector receive better prediction performance of MCC value than other single feature vectors. The probabilities of forming β-turn generated from SVM by these three feature vectors are gathered, and use as import information of the second layer SVM. The results were listed in Table 7.

Comparing the performance of double-layer SVM with only use single vector, PSSM, double-layer SVM of PSSM+SS could raise Q_{total} from 76.4% to 78.3%, and MCC value from 0.46 to 0.47. Besides $Q_{observed}$ slightly raise from 72.8% to 74.6%, double-layer SVM of PSSM+RSA-10 and single feature vector of PSSM almost get the same result with other performance measures. Comparing the result between SS and double-layer SVM of SS+RSA-10, after including RSA information, Q_{total} raise from 72.9% to 75.7%, $Q_{predicted}$ rise from 46.8% to 50.3%, $Q_{observed}$ decrease 76.5% to 68.4%, and the two MCC value are identical, 0.42. The predict performance between

double-layer SVM of PSSM+SS and PSSM+SS+RSA-10 are similar, and both are better than only import PSSM single vector. The achievements of PSSM+SS+RSA-10 are 78.7%, 55.4%, 67.8%, 0.47 in Q_{total}, $Q_{predicted}$, $Q_{observed}$, and MCC, respectively.

4. Discussion

It has been shown that, as using sequence or PSI-BLAST generated position-specific scoring matrix (PSSM) to predict β-turn in proteins, including secondary structure information would improve the prediction performance [14; 15]. As shown in Table 8., in this study, even only use secondary structure information generated by PSIPRED, the prediction performance MCC could achieve 0.42, which is only lower than MCC of BetaTPred2 (0.43) [16] and SVM(2005)(0.45) [35] (both are machine learning methods with PSSM and secondary structure information as import information), but higher than any other previous methods.

In this study, we found that relative solvent accessibility could provide useful information in β-turn prediction that has never been mentioned before. As only employing relative solvent accessibility as import information, the prediction performance of MCC could achieve 0.39, which is equal to MCC value of BTPRED. Although this MCC value is worse than three prediction methods, it is better than any other statistical base methods (Table 8..) As to the result of importing multiple feature vectors to SVM (Table. 6), comparing the performance between PSSM+SS+RSA-3 and PSSM+SS, while including RSA information, even though $Q_{observed}$ decreased, but the increment of $Q_{predicted}$ cause the raise of Q_{total}.

As shown in Table 5., comparing the result of two relative solvent accessibility coding scheme, the ten-classes method got better results in every performance measure than the three-classes one. Because β-turns tend to occur at solvent-exposed surface[4], it could be conjectured that the more classes in RSA prediction, the more information to represent the expose extent of a residue, and which could provide more helpful information for β-turn prediction.

Both PSIPRED predicted secondary structure and the Chou-Fasmsn conformational parameters could provide secondary structure information, but comparing with the result of these two feature vectors as listed in Table 5., the prediction performance of using Chou-Fasman parameters is worse than using PSIPRED predicted secondary structure. Maybe it is because the Chou-Fasmsn conformational parameters were calculated from specific protein set [8], it could only provide a general view of secondary structure information to each amino acid; but PSIPRED could give more limited secondary structure information in the light of specific residue. This idea could also be illustrated in comparing the result of relative solvent accessibility (RSA) with solvent exposed area (REA)(Table 8.). The solvent accessibility information used in this study was derived from Bordo and Argos [32]. The data was calculated from data taken from 55 proteins in the Brookhaven data base. In Table 2., the only clear trend is that some residues, such as R and K, locate themselves so that they have access to the solvent. The so-called hydrophobic residues, such as L and F, show no clear trend: they are found near the solvent as often as they are found buried. It also could provide a general view of the solvent exposure tendency of 20 amino acids only, but not in accordance with each residue in proteins. Thus tool-predicted RSA could provide more helpful information than SEA.

Several coding scheme in this study could get better prediction performance than any other previous method. The best performance of this study is import multiple feature vectors including multiple sequence alignment feature vector, secondary structure feature vector, and relative solvent accessibility feature vector (three classes). This approach yields superior results compared with existing method on the same dataset (Table 8.). Comparing the four performance measures with SVM(2005), only $Q_{observed}$ has almost the same value, other measures receive better result in this study:

Q_{total}, $Q_{predicted}$, $Q_{observed}$, and MCC are 79.6%, 57.4%, 66.1%, and 0.48 respectively in this work; and 77.3%, 53.1%, 67.0%, and 0.45 respectively in SVM(2005).

In conclusion, the β-turn prediction method described here yields predictions that are significantly more accurate than previous methods. Not only multiple sequence alignment and secondary structure information are important, relative solvent accessibility could also assist the prediction of β-turns.

5. Reference

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Table 1. Chou-Fasman conformational parameters

Amidio acid	P(a)	P(b)	P(turn)
Alanine	142	83	66
Arginine	98	93	95
Aspartic acid	101	54	146
Asparagine	67	89	156
Cysteine	70	119	119
Glumatic acid	151	37	74
Glutamine	111	110	98
Glycine	57	75	156
Histidine	100	87	95
Isoleucine	108	160	47
Leucine	121	130	59
Lysine	114	74	101
Methionine	145	105	60
Phenylalanine	113	138	60
Proline	57	55	152
Serine	77	75	143
Threonine	83	119	96
Tryptophan	108	137	96
Tyrosine	69	147	114
Valine	106	170	50

P(a), P(b) and P(turn) are conformational parameters of helix, β-sheet and β-turns.

Table 2. Solvent exposed area (SEA) of amino acids

Amino acid	SEA > 30 Å^2	SEA < 10 Å^2	30 > SEA > 10 Å^2
Serine	0.7	0.2	0.1
Threonine	0.71	0.16	0.13
Alanine	0.48	0.35	0.17
Glycine	0.51	0.36	0.13
Proline	0.78	0.13	0.09
Cysteine	0.32	0.54	0.14
Aspartic acid	0.81	0.09	0.1
Glumatic acid	0.93	0.04	0.03
Glutamine	0.81	0.1	0.09
Asparagine	0.82	0.1	0.08
Leucine	0.41	0.49	0.1
Isoleucine	0.39	0.47	0.14
Valine	0.4	0.5	0.1
Methionine	0.44	0.2	0.36
Phenylalanine	0.42	0.42	0.16
Tyrosine	0.67	0.2	0.13
Tryptophan	0.49	0.44	0.07
Lysine	0.93	0.02	0.05
Arginine	0.84	0.05	0.11
Histidine	0.66	0.19	0.15

Table 3. volume and hydropathy index of amino acids

amino acids	volume
G	60.1
A	88.6
V	140
L	166.7
I	166.7
M	162.9
F	189.9
Y	193.6
W	227.8
S	89
P	112.7
T	116.1
C	108.5
N	114.1
Q	143.8
K	168.6
H	153.2
R	173.4
D	111.1
E	138.4

Table 4. hydropathy index of amino acids

amino acids	Hydropathy index
G	-0.4
A	1.8
V	4.2
L	3.8
I	4.5
M	1.9
F	2.8
Y	-1.3
W	-0.9
S	-0.8
P	1.6
T	-0.7
C	2.5
N	-3.5
Q	-3.5
K	-3.9
H	-3.2
R	-4.5
D	-3.5
E	-3.5

Table 5. The prediction performance based on single feature vector

Coding scheme	Q_{total} (%)	$Q_{predicted}$ (%)	$Q_{observed}$ (%)	MCC
Sequence	71.9	44.2	54.4	0.30
PSSM	76.4	51.4	72.8	0.46
SS	72.9	46.8	76.5	0.42
RSA-3	69.4	41.7	62.3	0.30
RSA-10	72.9	46.5	69.8	0.39
C-F parameter	72.7	44.9	48.1	0.28
SEA	44.1	26.6	72.8	0.07
Volume	67.3	29.4	23.6	0.06
Hydropathy	59.0	30.5	52.5	0.12

Table 6. The prediction performance based on multiple feature vector

Coding scheme	Q_{total} (%)	$Q_{predicted}$ (%)	$Q_{observed}$ (%)	MCC
PSSM	76.4	51.4	72.8	0.46
PSSM+SS	78.2	54.3	71.9	0.48
PSSM+RSA-3	77.5	53.1	70.4	0.46
PSSM+RSA-10	77.8	53.9	67.6	0.45
PSSM+C-F	77.9	54.0	66.9	0.45
PSSM+SS+RSA-3	79.6	57.4	66.1	0.48

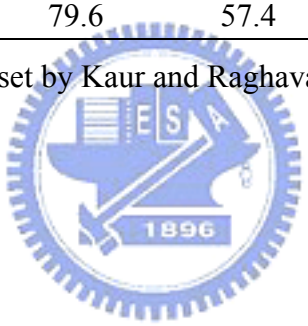
Table 7. The prediction performance based on multiple feature vector

Coding scheme	Q_{total} (%)	$Q_{predicted}$ (%)	$Q_{observed}$ (%)	MCC
PSSM	76.4	51.4	72.8	0.46
SS	72.9	46.8	76.5	0.42
RSA-10	72.9	46.5	69.8	0.39
PSSM+SS	78.3	54.6	69.4	0.47
PSSM+RSA-10	76.4	51.3	74.6	0.46
SS+RSA-10	75.7	50.3	68.4	0.42
PSSM+SS+RSA-10	78.7	55.4	67.8	0.47

Table 8. Comparing the prediction performance with previous methods

Method	Q_{total} (%)	$Q_{predicted}$ (%)	$Q_{observed}$ (%)	MCC
Chou-Fasman[a]	75.3	49.6	47.5	0.32
Thornton's[a]	75.2	49.3	44.9	0.31
GORBTURN[a]	75.4	49.6	37.7	0.28
1-4 & 2-3 correlation model[a]	74.8	48.0	39.8	0.28
Sequence coupled model[a]	75.4	49.6	40.0	0.28
BTPRED[a]	75.3	49.7	63.4	0.39
Nearest-neighbor	75.0	46.5	66.7	0.40
BetaTPred2	75.5	49.8	72.3	0.43
SVM (2005)	77.3	53.1	67.0	0.45
PSSM	76.4	51.4	72.8	0.46
SS	72.9	46.8	76.5	0.42
RSA-10	72.9	46.5	69.8	0.39
PSSM+SS	78.2	54.3	71.9	0.48
PSSM+SS+RSA-3	79.6	57.4	66.1	0.48

[a] Evaluated with the same dataset by Kaur and Raghava[14]



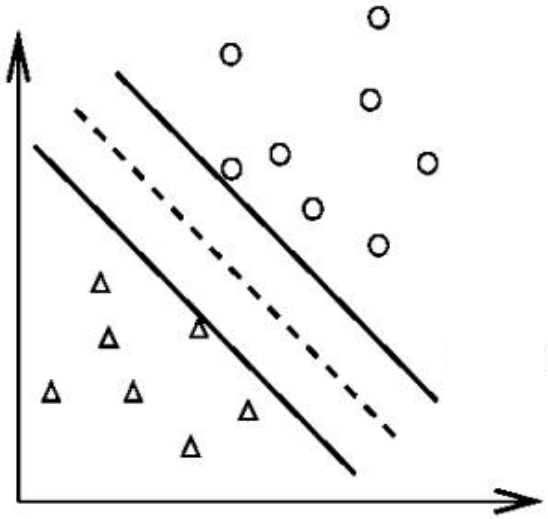


Figure 1. Optimal separating hyperplane(OSH) of SVM

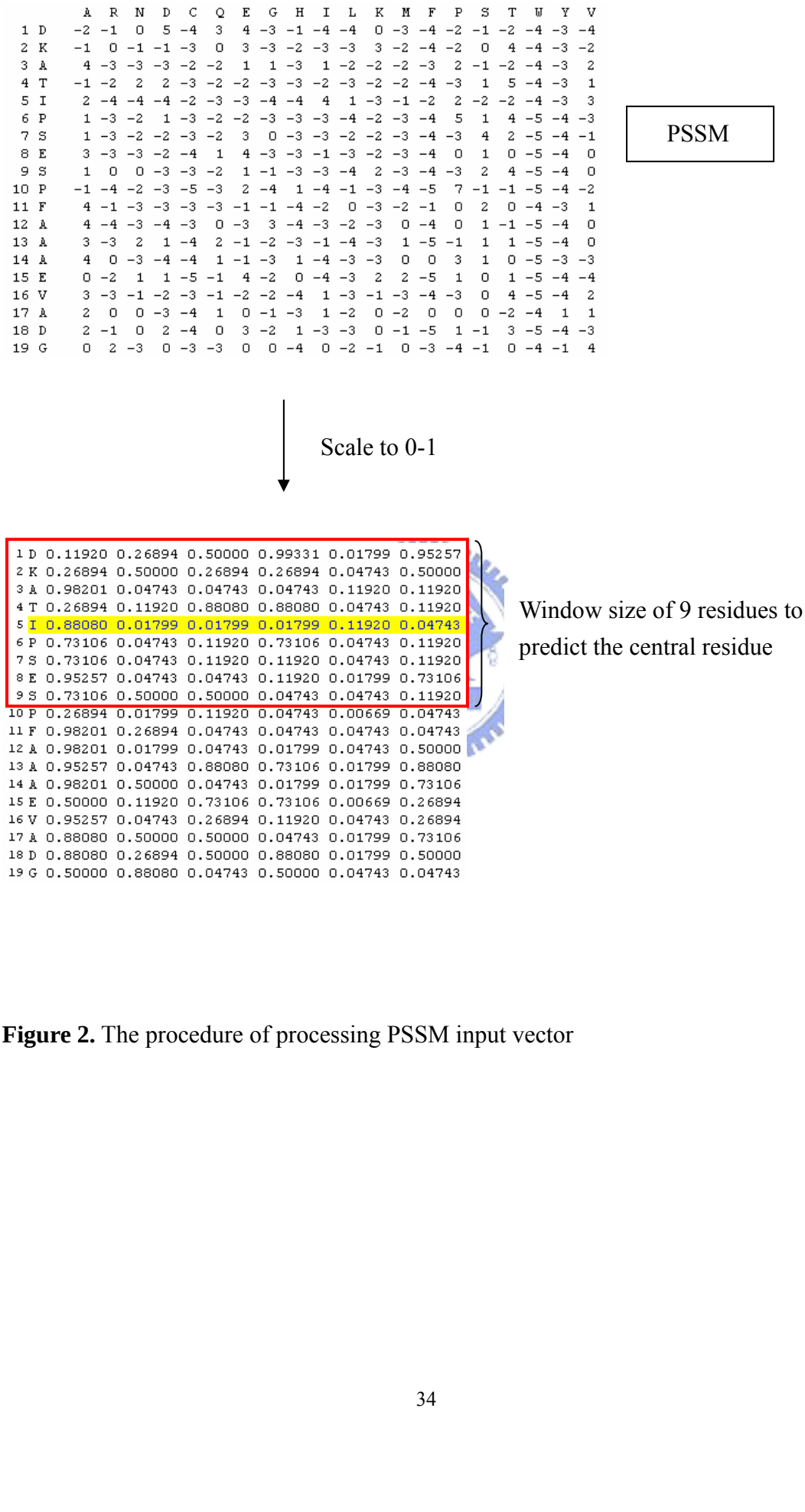


Figure 2. The procedure of processing PSSM input vector

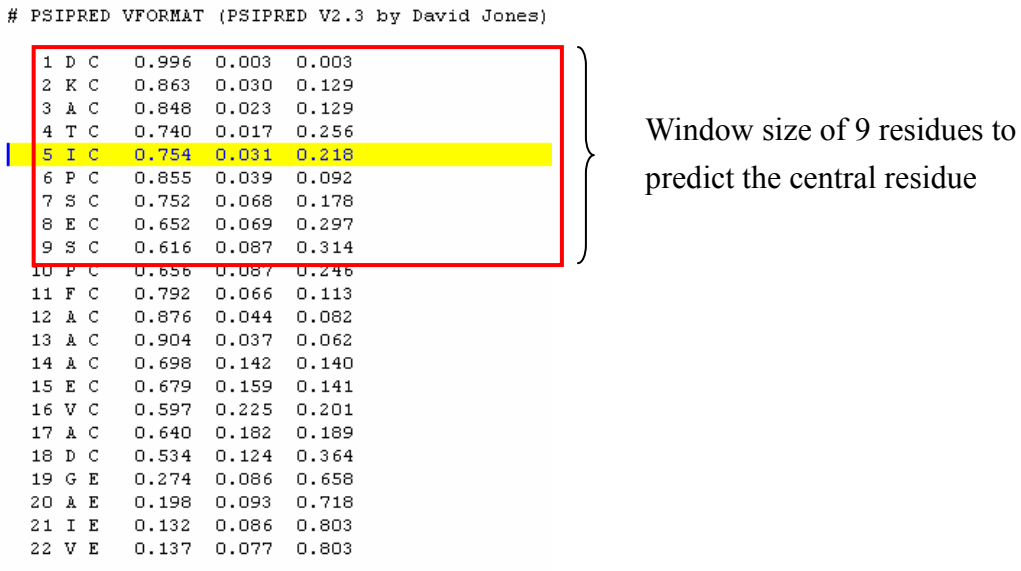
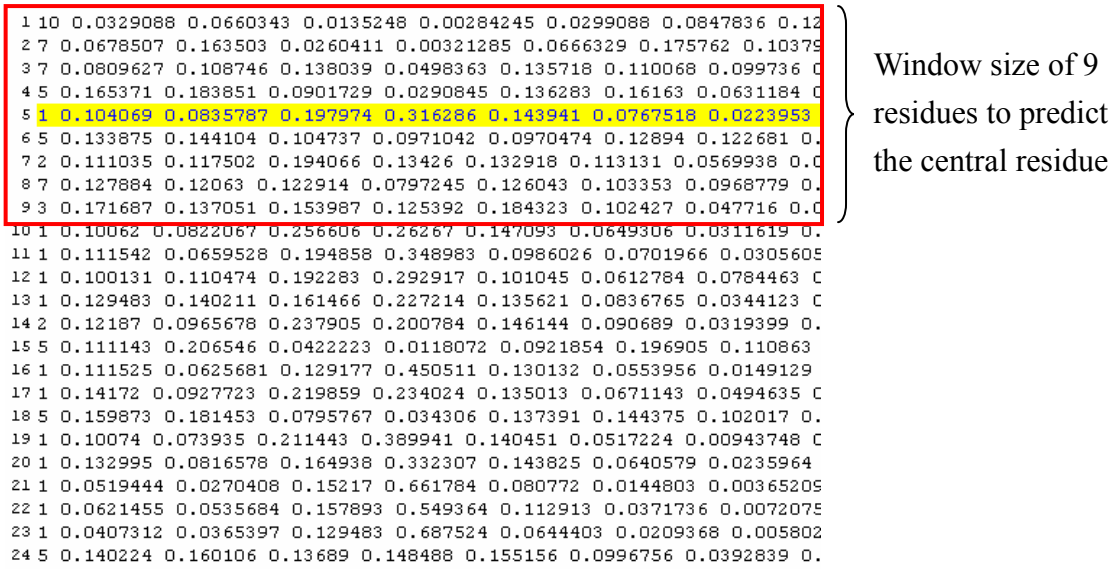


Figure 3. The procedure of processing secondary structure input vector



Window size of 9 residues to predict the central residue

Figure 4. The procedure of processing RSA-10 input vector

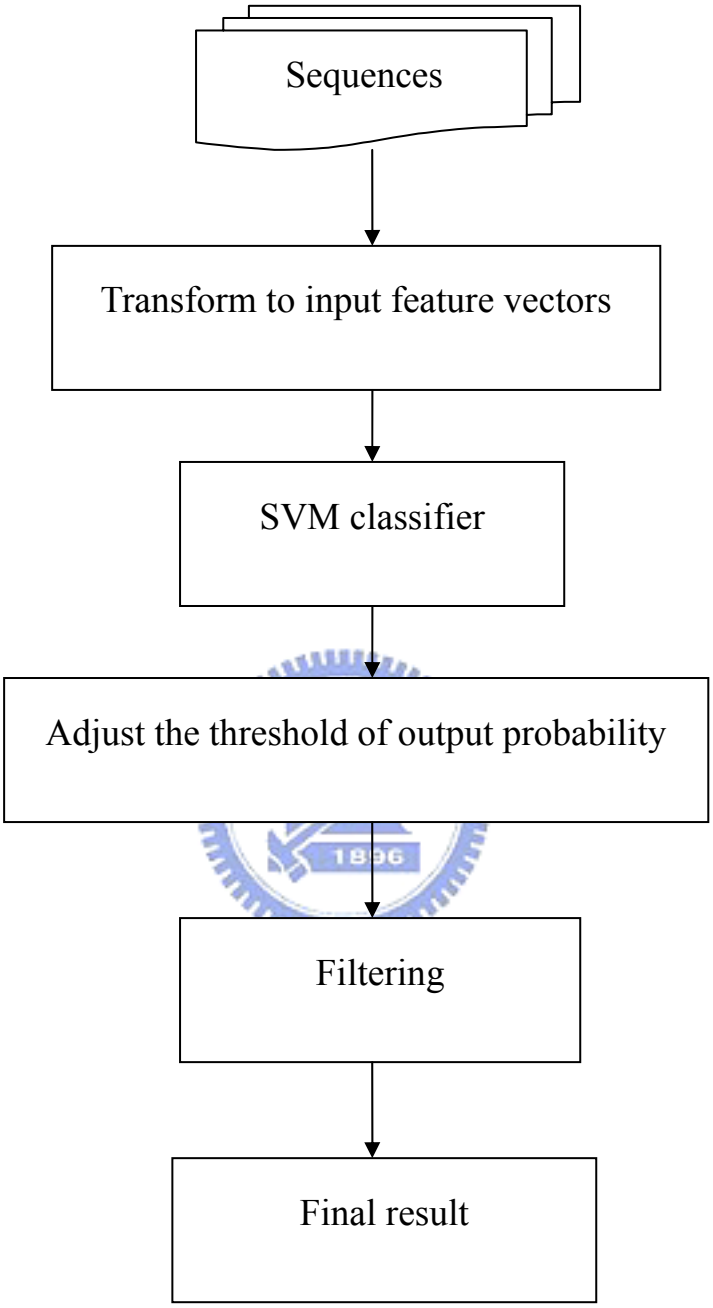


Figure 5. Simple flow chart of prediction β-turns using SVM in this study

Appendix 1. Types of β-turns

The Nine β-Turn Types Were Classified Based on a Total of 3899 Turns Extracted from the 205 Protein Chains (55)

Turn type	Number[a] of turns	Dihedral angles (°)[b]				$C^{\alpha}(i)$–$C^{\alpha}(i+3)$ distance (Å)
		$\phi(i+1)$	$\psi(i+1)$	$\phi(i+2)$	$\psi(i+2)$	
I	1231	−60	−30	−90	0	4.6
I′	127	60	30	90	0	4.6
II	405	−60	120	80	0	4.6
II′	90	60	120	−80	0	4.6
IV	1666	−61	10	−53	17	7.2
VIa1	15	−60	120	−90	0	3.4(8.8)[c]
VIa2	5	−120	120	−60	0	3.7(8.5)[c]
VIb	35	−135	135	−75	160	6.0(9.8)[c]
VIII	325	−60	−30	−120	120	6.3

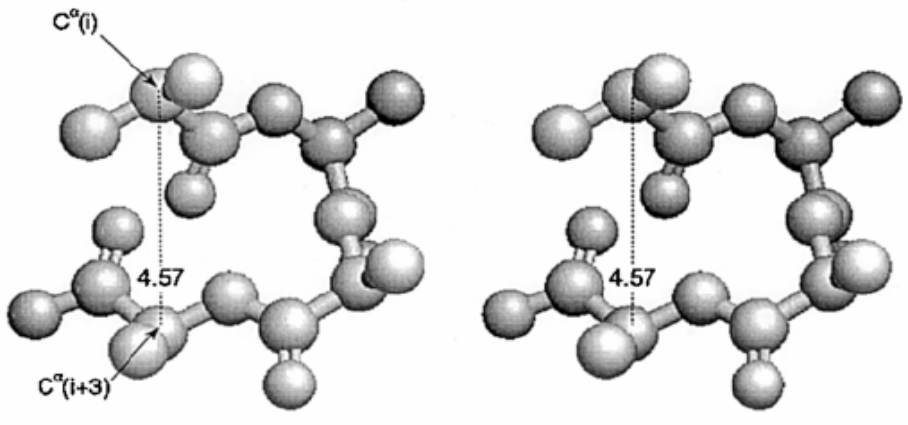
[a] Using normal cutoffs of 30° for deviation from standard angles, with one angle allowed to deviate by 45°.
[b] The idealized ϕ, ψ values as determined by Lewis *et al.* (44) except those for type IV; its ϕ, ψ values are the averaged values determined from the data set.
[c] For types VIa1, VIa2, and VIb, R_{i+2} must be a proline, and $\omega(i+1)$ should be 0° rather than 180° as in the other types; otherwise, the distance between $C^{\alpha}(i)$ and $C^{\alpha}(i+3)$ would be greater than 7.0 Å, as shown by the value in the parentheses.

* Chou, K. C. (2000). Prediction of tight turns and their types in proteins. *Anal Biochem.* 286, 1-16.

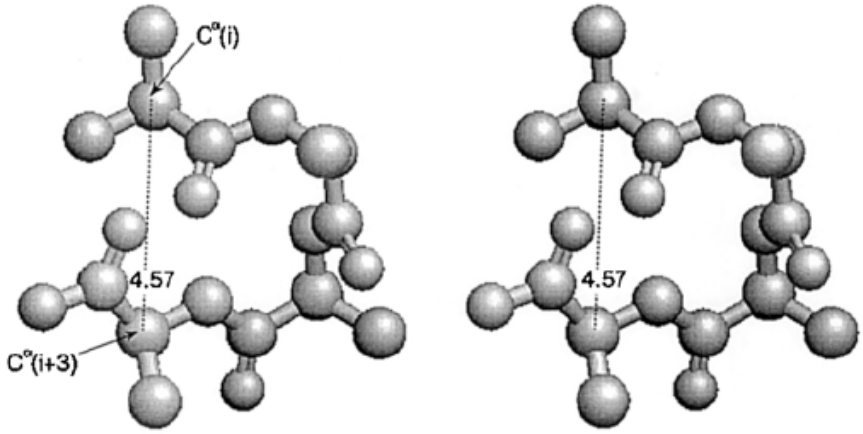
(55) Hutchinson, E. G., and Thornton, J. M. (1994) A revised set of potentials for β-turn formation in proteins. *Protein Sci.* **3,** 2207– 2216.

Appendix 1. (Continued)

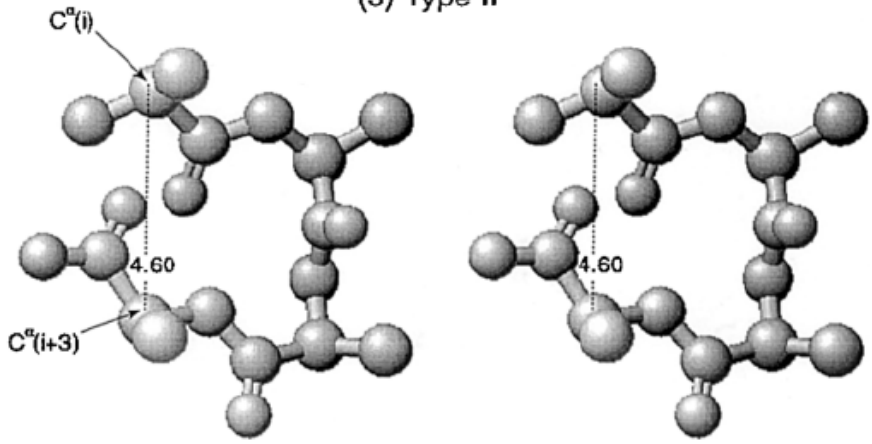
(1) Type I



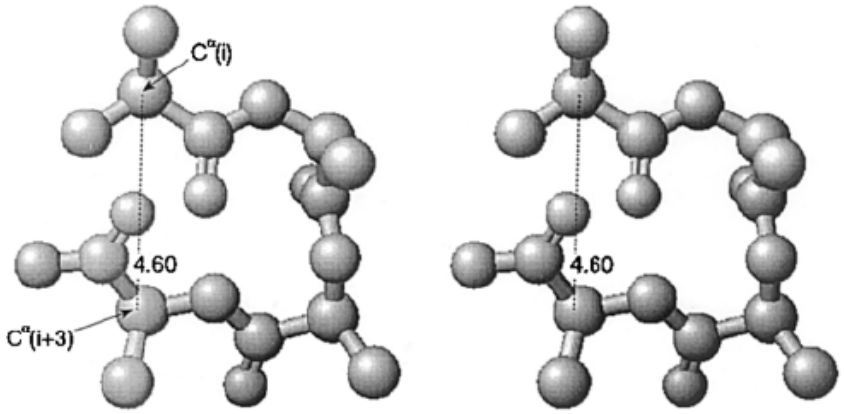
(2) Type I'



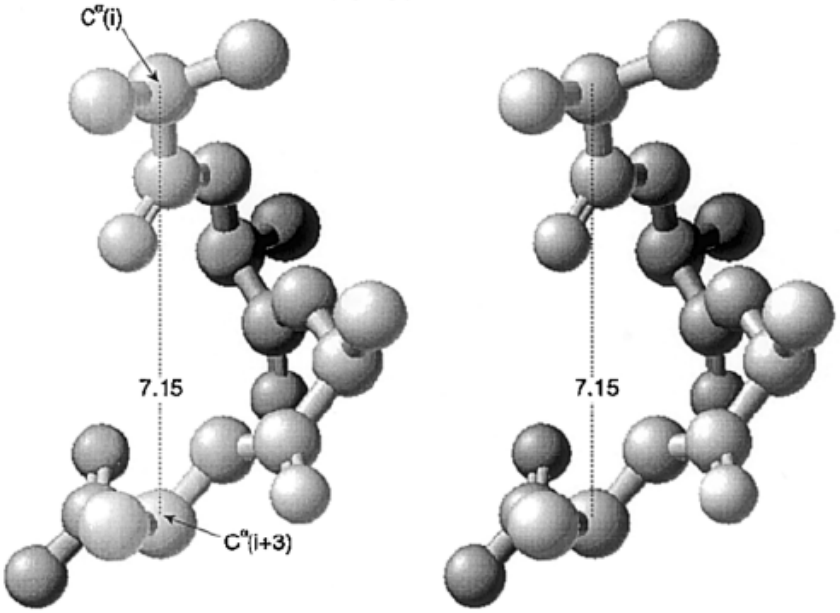
(3) Type II



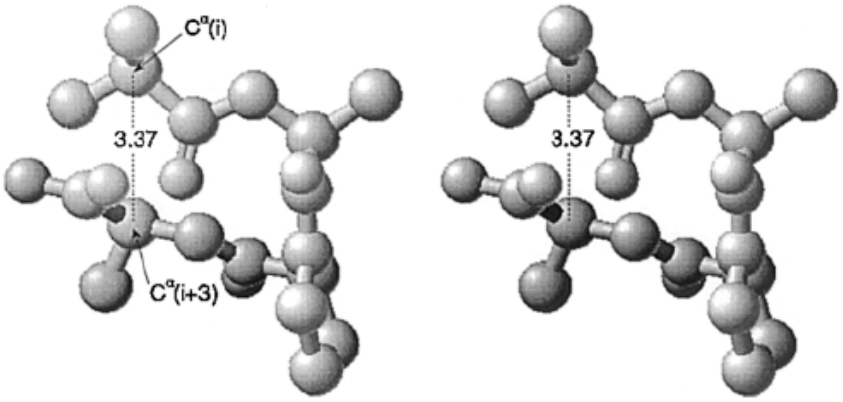
(4) Type II'

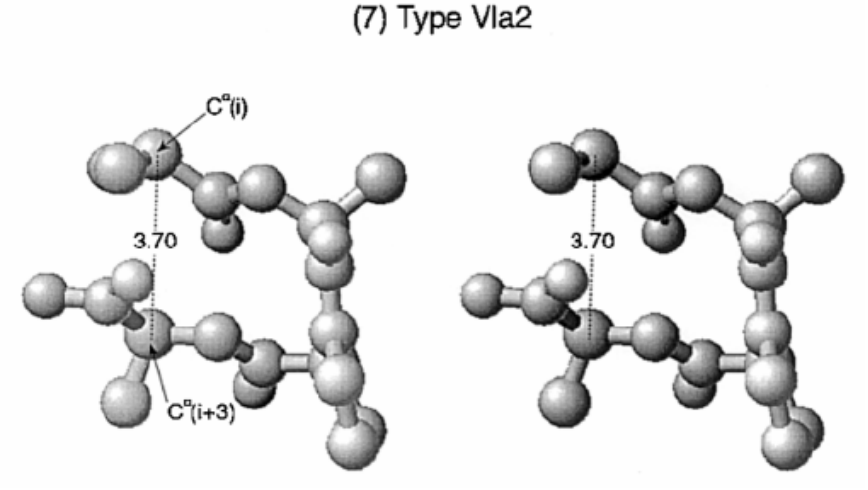


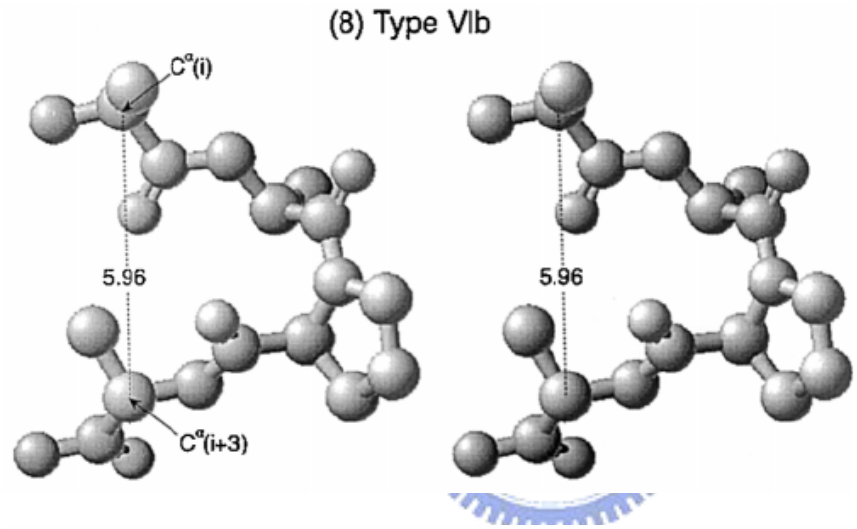
(5) Type IV

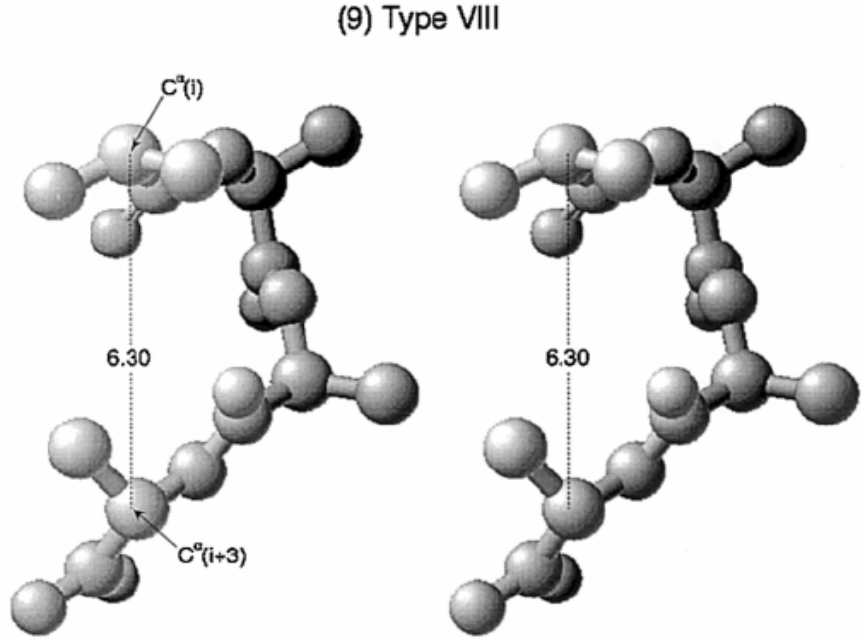


(6) Type VIa1









* Chou, K. C. (2000). Prediction of tight turns and their types in proteins. *Anal Biochem*. 286, 1-16.

Appendix 2. Protein chains (426) from Protein Data Bank used for β-turns analysis

119l	153l	1a1iA	1a1x	1a28B	1a2pA	1a2yA
1a2zA	1a34A	1a62	1a68	1a6q	1a7tA	1a8e
1a8i	1a9s	1aac	1aba	1ad2	1adoA	1af7
1afwA	1agjA	1agqD	1ah7	1aho	1aj2	1ajj
1ajsA	1ak0	1ak1	1ako	1akz	1al3	1alo
1alu	1alvA	1aly	1amm	1amp	1amuA	1amx
1anf	1aocA	1aohA	1aol	1aop	1aoqA	1aozA
1apyB	1aq0A	1aq6A	1aqb	1aqzB	1arb	1arv
1at0	1atlA	1atzB	1avmA	1awd	1awsA	1axn
1ayl	1azo	1ba1	1bbpA	1bdmB	1bdo	1bebA
1benB	1bfd	1bfg	1bftA	1bgc	1bgp	1bkf
1bkrA	1brt	1btkB	1btn	1bv1	1byb	1c52
1cbn	1cem	1ceo	1cewI	1cex	1cfb	1chd
1chmA	1ckaA	1clc	1cnv	1cpcB	1cpo	1cseE
1cseI	1csh	1csn	1ctj	1cydA	1dad	1dkzA
1dokA	1dorA	1dosA	1dun	1dupA	1dxy	1eca
1ecl	1ecpA	1ede	1edg	1edmB	1edt	1erv
1ezm	1fdr	1fds	1fit	1fleI	1fmtB	1fna
1fua	1furA	1fus	1fvkA	1fwcA	1g3p	1gai
1garA	1gd1O	1gdoA	1gifA	1gky	1gnd	1gotB
1gotG	1gsa	1guqA	1gvp	1ha1	1havA	1hcrA
1hfc	1hgxA	1hoe	1hsbA	1htrP	1hxn	1iakA
1idaA	1idk	1ido	1ifc	1igd	1iibA	1iso
1isuA	1ixh	1jdw	1jer	1jetA	1jfrA	1jpc
1kid	1knb	1kpf	1kptA	1kuh	1kveA	1kveB
1kvu	1kwaB	1lam	1latB	1lbu	1lcl	1lis
1lit	1lki	1lkkA	1lmb3	1lml	1lt5D	1ltsA
1lucB	1mai	1mbd	1mkaA	1mldA	1mml	1molA
1mpgA	1mrj	1mrp	1msc	1msi	1msk	1mtyB
1mtyD	1mtyG	1mucA	1mugA	1mwe	1mzm	1nar
1nbaB	1nbcA	1nciB	1neu	1nfn	1nif	1nls
1nox	1np1A	1npk	1nulB	1nwpA	1nxb	1ois
1onc	1onrA	1opd	1opy	1orc	1ospO	1ovaA
1oyc	1pcfA	1pda	1pdo	1pgs	1phe	1phnA
1php	1pii	1plc	1pmi	1pne	1pnkB	1poa
1poc	1pot	1ppn	1ppt	1prxB	1ptq	1pty

1pud	1qba	1qnf	1r69	1ra9	1rcf	1rec
1regY	1reqD	1rgeA	1rhs	1rie	1rmg	1rro
1rss	1rsy	1rvaA	1ryp1	1ryp2	1rypF	1rypI
1rypJ	1sbp	1sfp	1sftB	1sgpI	1skz	1sltA
1sluA	1smd	1spuA	1sra	1stmA	1svb	1svpA
1tadC	1tca	1tfe	1thv	1thx	1tib	1tif
1tml	1trkA	1tsp	1tvxA	1tys	1uae	1ubi
1uch	1unkA	1urnA	1uxy	1v39	1vcaA	1vcc
1vhh	1vid	1vif	1vin	1vjs	1vls	1vpsA
1vsd	1vwlB	1wab	1wba	1wdcA	1wer	1whi
1who	1whtB	1wpoB	1xgsA	1xikA	1xjo	1xnb
1xsoA	1xyzA	1yaiC	1yasA	1ycc	1yer	1ytbA
1yveI	1zin	256bA	2a0b	2abk	2acy	2arcA
2ayh	2baa	2bbkH	2bbkL	2bopA	2cba	2ccyA
2chsA	2ctc	2cyp	2dri	2end	2eng	2erl
2fdn	2fha	2fivA	2gdm	2hbg	2hft	2hmzA
2hpdA	2hts	2i1b	2ilk	2kinA	2kinB	2lbd
2mcm	2msbB	2nacA	2pgd	2phy	2pia	2pii
2plc	2por	2pspA	2pth	2rn2	2rspB	2sak
2scpA	2sicI	2sil	2sn3	2sns	2tgi	2tysA
2vhbB	2wea	3b5c	3chy	3cla	3cox	3cyr
3daaA	3grs	3lzt	3nul	3pcgM	3pte	3sdhA
3seb	3tss	3vub	4bcl	4mt2	4pgaA	4xis
5csmA	5hpgA	5icb	5p21	5pti	5ptp	6cel
6gsvA	7ahlA	7rsa	8abp	8rucI	8rxnA	